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Lab Resource: Stem Cell Line

Establishment of DYT5 patient-specific induced pluripotent stem cells with a *GCH1* mutation

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ABSTRACT

Peripheral blood mononuclear cells (PBMCs) were collected from a clinically diagnosed 20-year-old dystonia patient with a *GCH1* mutation (DYT5). Episomal vectors were used to introduce reprogramming factors (OCT3/4, SOX2, KLF4, L-MYC, LIN28, and p53 carboxy-terminal dominant-negative fragment) to the PBMCs. The generated iPSCs expressed pluripotency markers, and were capable of differentiating into derivates of all three germ layers *in vitro*. The iPSC line also showed a normal karyotype and preserved the *GCH1* mutation. This cellular model can provide opportunities to perform pathophysiological studies for aberrant dopamine metabolism-related disorders.

Resource table.

Unique stem cell line identifier	CIRAi001-A
Alternative name of stem cell line	HPS1816
Institution	Center for iPS Cell Research and Application (CiRA), Kyoto University
Contact information of distributor	Haruhisa Inoue haruhisa@cira.kyoto-u.ac.jp
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 20-year-old Sex: male
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Method of reprogramming	Episomal vectors
Associated disease	DYT5
Gene/locus	*GCH1*, c.626 + 1G>C
Method of modification	None
Gene correction	NO
Name of transgene or resistance	None
Inducible/constitutive system	None
Date archived/stock date	Oct 2016
Cell line repository/bank	RIKEN BioResource Center, Japan http://en.brc.riken.jp/index.shtml
Ethical approval	The Ethics Committee of the Department of Medicine and Graduate School of Medicine, Kyoto University (R0091 and G259) and Tokushima University (3172 and H26-9)

Resource utility

Research investigating dystonia using patient-derived iPSCs is limited, and a single nucleotide polymorphism in *GCH1* is reportedly associated with Parkinson's disease. This finding has the potential to provide new insight into not only DYT5 but also Parkinson's disease.

Resource details

Autosomal dominant GTP cyclohydrolase I (GCH1) deficiency, which is characterized by dopa-responsive dystonia with marked diurnal fluctuations, was first reported in 1976 by Segawa et al. (1976). GCH1 is involved in the production of tetrahydrobiopterin (BH4), which is an

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essential cofactor for biosynthesis of dopamine. Heterozygous *GCH1* mutation leads to partial deficiency of BH4 and selective decreases of tyrosine hydroxylase in nigrostriatal dopamine neurons, which causes dopa-responsive dystonia (Segawa et al., 2003), alternatively called as Segawa disease or DYT5. A large-scale genome-wide association study meta-analysis also revealed that a single nucleotide polymorphism at *GCH1* is associated with Parkinson's disease (Nalls et al., 2014). Peripheral blood mononuclear cells (PBMCs) derived from a 20-year-old dystonia patient with a *GCH1* mutation c.626+1G>C were reprogrammed by inducing episomal vectors with OCT3/4, SOX2, KLF4, L-MYC, LIN28, and p53 carboxy-terminal dominant-negative fragment, as previously reported (Okita et al., 2013) (Fig. 1A). The generated iPSCs expressed pluripotency markers, including NANOG and SSEA4 (Fig. 1B) and were capable of differentiating into derivates of all three germ layers, namely, endoderm (SOX-17), mesoderm (αSMA), and ectoderm (βIII-tubulin) (Fig. 1C). Short tandem repeat (STR) analysis of the iPSC line was identical to the parental PBMCs (Supplementary Table 1). The iPSC line had a normal karyotype (46 XY) (Fig. 1D). The reprogramming process did not alter a *GCH1* mutation, as demonstrated in Fig. 1E. By flow cytometry, 66.1% of iPSCs were positive for SSEA4 (Fig. 1F). Collectively, the HPS1816 iPSC line was karyotypically normal, pluripotent, and carried a *GCH1* mutation.

Materials and methods

Ethics statements

Generation and use of human iPSCs was approved by the Ethics Committee of the Department of Medicine and Graduate School of Medicine, Kyoto University, and Tokushima University. All methods were

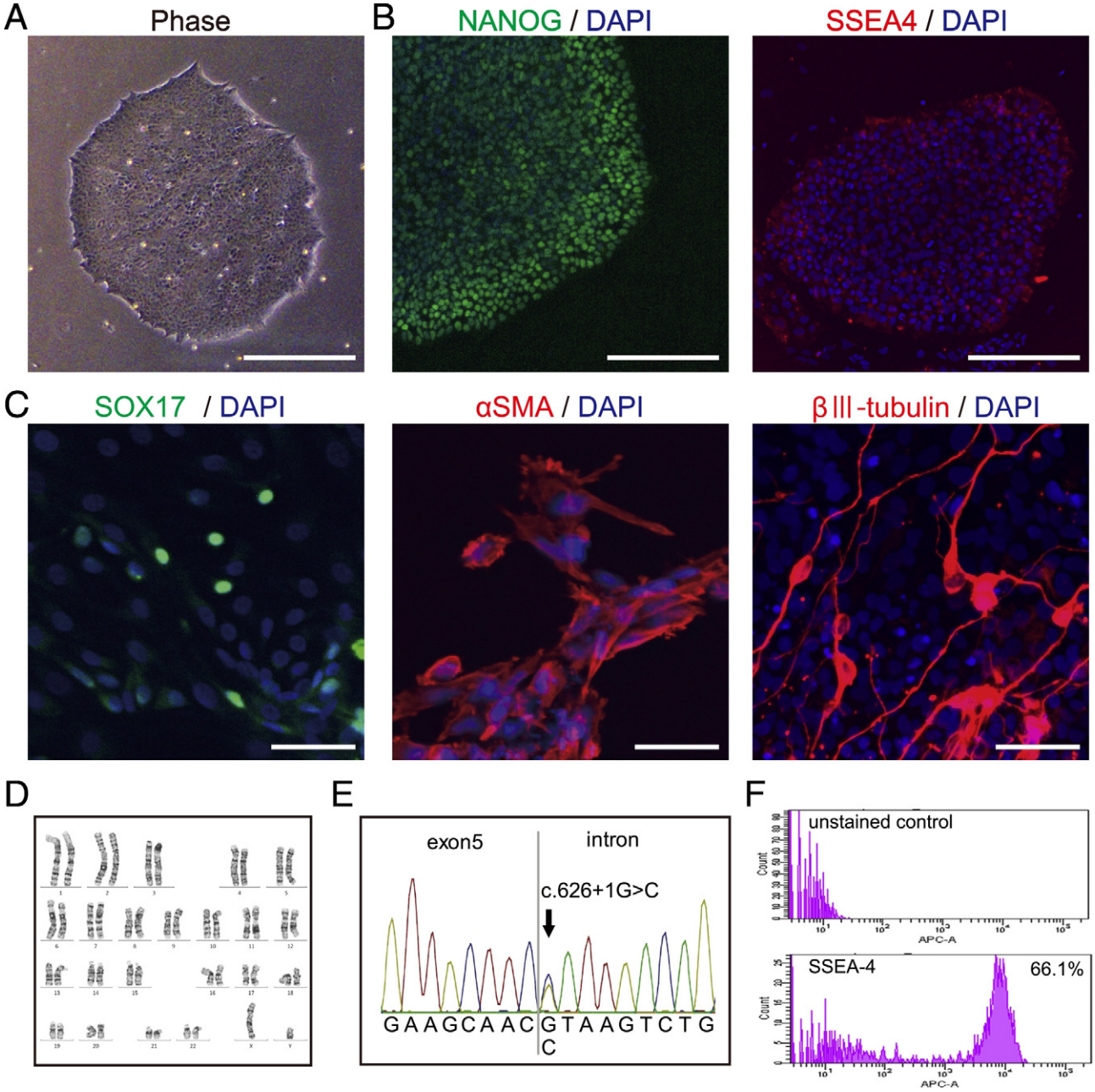

Fig. 1. Characterization of iPSC line. (A) The DYT5 patient iPSCs displayed a typical round-shaped colony under microscopy. Scale bar: 200 μm (B) Immunocytochemical staining of iPSCs showed high expression of pluripotency marker NANOG and SSEA4. Scale bars: 200 μm. (C) Pluripotency of iPSCs was confirmed by *in vitro* three-germ layer assay. Scale bars: 50 μm. (D) Karyotype analysis of patient iPSCs showed a normal karyotype of 46 XY. (E) Sanger sequence of the *GCH1* gene in iPSCs showed a heterozygous c.626+1G>C substitution. (F) FACS analysis with evaluation of SSEA-4 staining.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Assess staining of pluripotency markers: NANOG and SSEA4	Fig. 1 panel B
	Flow cytometry	SSEA4 66.1%	Fig. 1 panel F
Genotype	Karyotype (G-banding) and resolution	46XY Resolution 300–400	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	16 loci, matched	Supplementary Table 1
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous c.626 + 1G>C mutation	Fig. 1 panel E
	Southern Blot OR WGS	Not performed	Not performed
Microbiology and virology	Mycoplasma	Contamination of Mycoplasma was negative	Not shown but available from author
Differentiation potential	Embryoid body formation OR Teratoma formation OR Scorecard	Describe expression of genes in embryoid bodies: SOX-17, αSMA, and βIII-tubulin	Fig. 1 panel C
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	HIV1 (negative), HIV2 (not performed), Hepatitis B (negative), and Hepatitis C (negative)	Not shown but available from author
Genotype additional info	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

performed in accordance with approved guidelines. Formal informed consent was obtained from the patient.

Generation of iPSCs

Human complementary DNAs for reprogramming factors were transduced in PBMCs with episomal vectors (OCT3/4, SOX2, KLF4, L-MYC, LIN28 and p53 carboxy-terminal dominant-negative fragment). The generated iPSCs were grown under feeder-free conditions on laminin-511 E8 (Nippi, Tokyo, Japan)-coated plates with StemFit (AK02N or AK03N, Ajinomoto, Tokyo, Japan) (Nakagawa et al., 2014) (Table 1).

Karyotyping and genotyping of GCH1

Karyotype analysis was performed by LSI Medience (Tokyo, Japan). Genotyping of the *GCH1* mutation was performed by PCR amplification of genomic DNA and direct sequencing (3500xL Genetic Analyzer, Applied Biosystems, Waltham, MA) (Table 2).

In vitro three-germ layer assay

iPSCs were dissociated with 0.5× TrypLE Select (Gibco, Waltham, MA) and used for embryoid body (EB) formation. Cells were transferred to suspension plates in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12, Sigma-Aldrich, St. Louis, MO) containing 20% knockout serum replacement (KSR, Life Technologies, Waltham, MA), 2 mM L-glutamine, 0.1 mM non-essential amino acids (NEAA, Invitrogen, Waltham, MA), 0.1 mM 2-mercaptoethanol (2-ME, Life Technologies), and 0.5% penicillin and streptomycin. On Day 8, EBs were seeded onto gelatin-coated plates, and cultivated for 8 days.

Immunocytochemical staining

Cells were fixed in 4% paraformaldehyde in PBS for 20 min at room temperature, washed three times with PBS after each step, and blocked with 5% bovine serum albumin for 1 h at room temperature followed by incubation with primary antibodies at 4 °C overnight. The primary antibodies used for staining are listed in Table 2. Suitable secondary antibodies were incubated at room temperature for 1 h. Nuclear staining was also performed with DAPI for 5 min. Images were collected using BIOREVO BZ-9000 (Keyence, Osaka, Japan) or Olympus CKX41 (Olympus, Tokyo, Japan) (Tables 1 and 2).

Flow cytometry analysis

iPSCs were dissociated into single cells using Accumax (Innovative Cell Technologies, San Diego, CA) and were incubated at 1.0×10^6 cells/-ml in PBS with 2% FBS and 20 μl SSEA4 APC conjugated monoclonal antibody (BD Biosciences, Franklin Lakes, NJ) for 30 min at 4 °C. After staining, the cells were washed twice in PBS with 2% FBS, and then stored in PBS with 2% FBS on ice. Cells were analyzed on a FACS Aria II (BD Biosciences) high-speed cell sorter using a 647 nm excitation

Table 2
Summary of antibodies and primers.

Antibodies used for immunocytochemistry/flow cytometry			
	Antibody	Dilution	Company Cat# and RRID
Pluripotency markers	Rabbit anti-NANOG	1:500	Cosmo Bio Co Cat# REC-RCAB0003P, RRID: AB_1962353
Pluripotency markers	Mouse anti-SSEA4	1:1000	Millipore Cat# MAB4304, RRID: AB_177629
Differentiation markers	Goat anti-SOX-17	1:1000	R and D Systems Cat# AF1924, RRID: AB_355060
Differentiation markers	Mouse anti-αSMA	1:500	Dako Cat# M0851, RRID: AB_2223500
Differentiation markers	Rabbit anti-βIII-tubulin	1:2000	Cell Signaling Technology Cat# 5568P, RRID: AB_10692510
Secondary antibodies	Donkey anti-Goat IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11055, RRID: AB_142672
Secondary antibodies	Goat anti-Rabbit IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11034, RRID: AB_2576217
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-11030, RRID: AB_2534089
Secondary antibodies	Goat anti-Rabbit IgG Alexa Fluor 546	1:1,000	Thermo Fisher Scientific Cat# A-11010, RRID: AB_10584649
Primers			
	Target	Forward/reverse primer (5′-3′)	
Genotyping	*GCH1* exon 1	CAGGTGCAGCAATGGGTTCC/CGCACTGACCTGAGATGGTCTCC	
Genotyping	*GCH1* exon 2	TTCTCCTTCCTCTTCCATACTGC/TGAGAGCCTTCTGCTACTTTGG	
Genotyping	*GCH1* exon 3	CCGCATTTACCAACGGACAAC/CAGGTAAAGAGAGAAAGCCTGATG	
Genotyping	*GCH1* exon 4	TTCCTCTTGCAGCCCACTTGCTTC/CACAGTGGCTCATGCCTGTAATCC	
Genotyping	*GCH1* exon 5	GGGCTTCAGGGTGTTCTGAGA/AGCTTTAGGCTCAGGGATGG	
Genotyping	*GCH1* exon 6	ACTTGGTAACTGTGAGCTGAG/GGTGGCAAGAAGAAAGTAGAGG	

wavelength and 100 μm nozzle. Unstained cells were also analyzed as negative controls to exclude data from non-specific fluorescence.

DNA fingerprinting

STR analysis was performed by BEX (Tokyo, Japan) from PBMCs and the iPSC line.

Mycoplasma test

iPSCs were confirmed to be mycoplasma-negative using the MycoAlert kit (Lonza, Basel, Switzerland) in accordance with the manufacturer's instructions.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.07.029.

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